



TITLE:

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AUTHOR(S):

Ando, Katsutoshi; Okada, Yoshinori; Akiba, Miki; Kondo, Takashi; Kawamura, Tomohiro; Okumura, Meinoshin; Chen, Fengshi; ... Yokoyama, Akihito; Hoshino, Tomoaki; Watanabe, Kentaro

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RESEARCH ARTICLE

# Lung Transplantation for Lymphangioleiomyomatosis in Japan

Katsutoshi Ando<sup>1</sup>, Yoshinori Okada<sup>2</sup>, Miki Akiba<sup>2</sup>, Takashi Kondo<sup>2</sup>, Tomohiro Kawamura<sup>3</sup>, Meinoshin Okumura<sup>3</sup>, Fengshi Chen<sup>4</sup>, Hiroshi Date<sup>4</sup>, Takeshi Shiraishi<sup>5</sup>, Akinori Iwasaki<sup>5</sup>, Naoya Yamasaki<sup>6</sup>, Takeshi Nagayasu<sup>6</sup>, Masayuki Chida<sup>7</sup>, Yoshikazu Inoue<sup>8,10</sup>, Toyohiro Hirai<sup>9,10</sup>, Kuniaki Seyama<sup>1,10\*</sup>, Michiaki Mishima<sup>9,10</sup>, Respiratory Failure Research Group of the Japanese Ministry of Health, Labour, and Welfare<sup>†</sup>

**1** Division of Respiratory Medicine, Juntendo University, Faculty of Medicine and Graduate School of Medicine; 2-1-1 Hongo; Bunkyo-Ku; Tokyo, Japan, **2** Department of Thoracic Surgery, Institute of Development, Aging and Cancer, Tohoku University; Seiryomachi 4-1, Aoba-ku Sendai, Miyagi, Japan, **3** Department of General Thoracic Surgery, Osaka University Graduate School of Medicine; 2-2 Yamadaoka, Suita, Osaka, Japan, **4** Department of Thoracic Surgery, Kyoto University Graduate School of Medicine, 54 Shogoin-kawahara-cho, Sakyo-ku, Kyoto, Japan, **5** Department of General Thoracic, Breast, and Pediatric Surgery, Fukuoka University School of Medicine; 7-45-1 Nanakuma, Jonan-ku, Fukuoka City, Fukuoka, Japan, **6** Division of Surgical Oncology, Department of Translational Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences; 1-7-1 Sakamoto, Nagasaki, Japan, **7** Department of General Thoracic Surgery, Dokkyo Medical University; 880 Kitakobayashi, Mibu-machi, Shimotsuga-gun, Tochigi, Japan, **8** Clinical Research Center, National Hospital Organization Kinki-Chuo Chest Medical Center; 1180, Nagasonecho, Kita-Ku, Sakai, Osaka, Japan, **9** Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University; Kawahara 54, Shogoin, Sakyo-ku, Kyoto, Japan, **10** Respiratory Failure Research Group from the Ministry of Health, Labour and Welfare, Japan, (Office) Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University; Kawahara 54, Shogoin, Sakyo-ku, Kyoto, Japan

<sup>†</sup> Membership of the Respiratory Failure Research Group of the Japanese Ministry of Health, Labour, and Welfare is provided in the Acknowledgments.

\* [kseyama@juntendo.ac.jp](mailto:kseyama@juntendo.ac.jp)



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## Abstract

### Background

Lung transplantation has been established as the definitive treatment option for patients with advanced lymphangioleiomyomatosis (LAM). However, the prognosis after registration and the circumstances of lung transplantation with sirolimus therapy have never been reported.

### Methods

In this national survey, we analyzed data from 98 LAM patients registered for lung transplantation in the Japan Organ Transplantation Network.

### Results

Transplantation was performed in 57 patients as of March 2014. Survival rate was 86.7% at 1 year, 82.5% at 3 years, 73.7% at 5 years, and 73.7% at 10 years. Of the 98 patients, 21 had an inactive status and received sirolimus more frequently than those with an active history (67% vs. 5%,  $p < 0.001$ ). Nine of twelve patients who remained inactive as of March

2014 initiated sirolimus before or while on a waiting list, and remained on sirolimus thereafter. Although the statistical analysis showed no statistically significant difference, the survival rate after registration tended to be better for lung transplant recipients than for those who awaited transplantation ( $p = 0.053$ ).

## Conclusions

Lung transplantation is a satisfactory therapeutic option for advanced LAM, but the circumstances for pre-transplantation LAM patients are likely to alter with the use of sirolimus.

## Introduction

Lymphangioleiomyomatosis (LAM) is a rare neoplastic disease, characterized by the proliferation of abnormal smooth muscle-like cells (LAM cells), which lead to cystic destruction of the lungs, chylous effusions and the formation of lymphangioleiomyomas [1]. This disease is found primarily in women of childbearing age and can occur either as a sporadic disease (sporadic LAM) or as a pulmonary manifestation of tuberous sclerosis complex (TSC) (TSC-associated LAM) [2–4]. Clinical manifestations include exertional dyspnea, pneumothorax, hemoptysis, and chylous leakage into the pleural and/or peritoneal cavities. The disease continuously progresses and eventually results in respiratory failure, although the pace of progression varies considerably among LAM patients [5]. Until recently, therapeutic medical interventions for LAM were very limited; that is, only lung transplantation has been established as the definitive treatment option for patients with an advanced stage of LAM [6]. Owing to its rarity, LAM accounted for only about 1.0% of all causes for whole heart or lung transplantations in an international survey [7]. However, in Japan, advanced LAM is one of the main indicators (20%) requiring such grafts. Other candidates are sufferers with primary pulmonary hypertension (18%) or idiopathic interstitial pneumonia (18%); these patients are the most common recipients of single lung transplants from brain-dead-donors [8].

Recently, a molecular-targeting therapy for LAM has been established. LAM cells appear to result from the dysregulated mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) signaling, which is a key regulatory pathway of protein synthesis, cell growth, and energy metabolism due to the TSC gene mutation [2–4]. Sirolimus, an mTOR inhibitor, blocks mTORC1-mediated activation and restores homeostasis [9]. A recent clinical trial, the Multi-center International Lymphangioleiomyomatosis Efficacy and Safety of Sirolimus (MILES) trial, successfully demonstrated that sirolimus stabilized lung function and improved the quality of life in patients with LAM [10]. Furthermore, our clinical review of Japanese LAM patients indicated that low-dose sirolimus yielded the clinical benefits of improving pulmonary function and decreasing chylous effusion [11]. Although sirolimus had been administered as off-label in Japan if LAM patients wish to take, its use has been approved for LAM therapy and covered by medical insurance in Japan as of December 2014. That is, sirolimus is predicted to become a common treatment strategy that will provide a huge impact on lung transplantation for LAM patients not only in Japan but also worldwide. In fact, the benefit seems to be greatest in Japan, since transplantation for LAM patients is the most frequent among various indications of underlying pulmonary diseases in Japan [8]. Therefore, the national survey conducted here to elucidate the clinical features of LAM in patients with an advanced stage of the disease and to document their outcomes after registration for lung transplantation provides a much-needed basis for foreseeing the future of this procedure in Japan.

## Materials and Methods

### Study design

From 1999 to 2013, 98 LAM patients from six lung transplant centers were registered for lung transplantation in the Japan Organ Transplantation Network. To determine their clinical characteristics at registration and their post-transplant status as of March 2014, we retrospectively reviewed their medical records and summary worksheets at registration. The data compiled included presenting features, diagnostic methods, clinical findings, pre-transplant history of sirolimus treatment, results of arterial blood gas analysis, echocardiography, right heart catheterization (RHC), six-minute walk test, and lung function tests. Post-transplant outcomes including LAM-related complications in one year after transplantation, survival times, and causes of death were analyzed. We noted the causes of deaths after lung transplantation as well as while on the waiting list.

RHC was performed at registration in 25 (25.5%) of 98 patients. The diagnosis of pre-capillary pulmonary hypertension (PH) was established based on data of RHC; which was defined as mean pulmonary artery pressure (MPAP)  $\geq 25$  mmHg and pulmonary artery wedge pressure (PAWP)  $\leq 15$  mmHg [12]. Our retrospective review, verbal consent procedure and analysis of their data in study, was approved by the ethics committee of Juntendo, Tohoku, Osaka, Kyoto, Fukuoka and Nagasaki Universities, and all patient data were anonymous. None of the transplant donors were from a vulnerable population and all donors or next of kin provided written informed consent that was freely given.

### Inactive system

The Japanese organ-transplant registration system allows recipients to self-determine whether they wish to have an “inactive status”, i.e., temporary removal from the waiting list for lung transplantation; when they prefer restoration to an “active status”, they can resume their position on the waiting list where they were originally registered. Common reasons for an inactive status to postpone transplantation include: 1) the prospective recipient has been stable with medical care although severely disabled, and 2) the recipient is anxious and fears the operation. To determine the characteristics of patients who choose an inactive status, we explored the specific reasons for being an inactive status from medical records, classified all 98 patients as with or without that history, and then compared their clinical data.

### Lung function tests

Lung function tests were performed in each institution according to American Thoracic Society standards. Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV<sub>1</sub>), diffusing capacity for carbon monoxide (DL<sub>CO</sub>) and DL<sub>CO</sub> per unit of alveolar volume (DL<sub>CO</sub>/VA) for each patient were expressed as a percentage of the predicted values (FVC %predicted, FEV<sub>1</sub> %predicted, DL<sub>CO</sub> %predicted and DL<sub>CO</sub>/VA %predicted). The reference values were obtained from the prediction equations of the Japanese Respiratory Society [13].

### Statistical analysis

We used the *Chi*-squared or Mann-Whitney tests, where appropriate, to compare patients' characteristics. Correlations of MPAP with clinical parameters were evaluated by Spearman's rank correlation analysis. Survival rates were estimated using the Kaplan-Meier method. For all statistical analyses, we used the SPSS, version 21, and a *p* value less than 0.05 was considered significant.

## Results

### Patients' characteristics at the registration for lung transplantation

All patients were female, and their mean ages at the onset of symptoms and at the diagnosis were 32.1 and 34.2 years, respectively. Ninety patients (92%) had been diagnosed with sporadic LAM, whereas eight had TSC-associated LAM. The diagnosis of LAM was established by histopathological examination ( $n = 81$ ), by cytological examination of chylous fluid ( $n = 1$ ), or by the combined findings of characteristic computed tomography (CT) and/or elevated serum VEGF-D ( $> 800$  pg/mL) ( $n = 8$ ) (see [S1 Table](#) for detailed clinical characteristics at the diagnosis of LAM).

The mean age at registration for lung transplantation was 39.5 years ([Table 1](#)); the mean period from diagnosis to registration was 5.3 years. At registration, 21 patients (21%) had chylous effusion; nine had both chylothorax and ascites, whereas eight had chylothorax alone and four had chylous ascites alone. Twenty-two patients (22%) had renal angiomyolipomas. Lymphangiomyomas along axial lymphatics were suspected from imaging studies of fifty patients (51%): 14 (14%) had abdominal lymphadenopathies, two (2%) had pelvic lymphadenopathies, and 34 (35%) had both abdominal and pelvic lymphadenopathies.

All patients were severely disabled at the time of registration. More than half ( $56/98 = 57\%$ ) showed dyspnea of modified MRC grade III or more. As [Table 1](#) depicts, mean values of FVC, FEV<sub>1</sub>, DL<sub>CO</sub>, PaO<sub>2</sub>, and six-minute-walking distance (6MWD) at registration were 2.11 L (70.3%predicted), 0.85 L (32.8%predicted), 24.7%predicted, 55.7 Torr, and 252 m, respectively. Ninety-four patients (96%) utilized supplemental oxygen at registration.

### Haemodynamics at registration for lung transplantation

Data from echocardiography were available for all patients, and 16 (16%) showed an estimated systolic pulmonary arterial pressure (SPAP)  $\geq 40$  mmHg ([Table 2](#)). The results of RHC in 25 patients yielded mean values for SPAP, MPAP and pulmonary vascular resistance (PVR) of 31.5 mmHg, 22.8 mmHg and 256.0 dyne sec/cm<sup>5</sup>, respectively. Nine of 25 patients (36%) had MPAP  $\geq 25$  mmHg, and pre-capillary PH was diagnosed in eight patients (one patient had PAWP  $\geq 15$  mmHg). The correlation between MPAP and clinical parameters was examined in 25 LAM patients who underwent RHC ([S2 Table](#)). MPAP had a statistically significant negative correlation with DL<sub>CO</sub> %predicted ( $r = -0.654$ ,  $p = 0.001$ ), but not with other clinical parameters including PaO<sub>2</sub> ( $r = -0.348$ ,  $p = 0.223$ ) and FEV<sub>1</sub> %predicted ( $r = 0.283$ ,  $p = 0.181$ ). Among 25 LAM patients who had RHC at registration, the actual values of SPAP estimated by echocardiography were available in only 13 patients; results for the remaining 12 patients were just descriptions, e.g., "no pulmonary hypertension." When the estimated SPAP at echocardiography and MPAP at RHC in the 13 patients were analyzed, we found a statistically significant correlation between them ([S1 Fig](#);  $r = 0.589$ ,  $p = 0.034$ ). All six patients with estimated SPAP  $\geq 40$  mmHg at echocardiography also had MPAP  $\geq 25$  mmHg at RHC.

### Lung transplantation

The status of 98 LAM patients (as of March 2014) who were registered for lung transplantation is summarized in [Table 3](#). Transplantation was performed in 57 patients (58%); 53 patients received lungs from brain-dead-donors (single lung transplantation in 48 patients and double in 5 patients), whereas four received lobar lungs from living-donors. Mean ages at registration and at transplantation were 39.0 and 41.8 years, respectively. Eight of 57 patients (14%) who were transplanted had a history of inactive status while they waited: i.e., an inactive period

(median) of 170 days (range; 48–1,624 days). Duration from registration to transplantation (median) was 969 days (range; 29–3,381 days).

Thirty-two patients (33%) were still on the waiting list; twenty patients (20%) were in active status (the median waiting period after registration, 414 days), whereas twelve (12%) remained in the inactive group (the median waiting period after registration, 2,153 days). Nine patients (9%) died while on the waiting list (313 days after registration [median]), and one of them had a history of inactive status (Table 3).

**Table 1. Clinical characteristics of 98 LAM patients at registration for lung transplantation**

Variable	Value
Age at registration for lung transplantation -yr (range)	39.5 ± 7.3 (24–59)
Clinical features at registration—N. (%)	
Patients with lung involvement alone	28 (29)
Patients with extrapulmonary manifestation	70 (71)
Chylous effusion	21 (21)
Chylothorax	17 (17)
Ascites	13 (13)
Mediastinal lymphadenopathy	7 (7)
Angiomyolipoma	22 (22)
Retroperitoneal lymphangiomyoma	48 (49)
Pelvic lymphangiomyoma	36 (37)
Lymph edema	6 (6)
Body mass index—(range)	19.2 ± 3.6 (13.4–29.3)
Modified MRC classification -N (%)	
I	1 (1)
II	41 (42)
III	47 (48)
IV	8 (8)
Unknown	1 (1)
Pulmonary function (range)	
FVC (L)	2.11 ± 0.72 (0.57–3.88)
FVC (%predicted)	70.3 ± 24.3 (18.5–124.7)
FEV <sub>1</sub> / FVC	40.2 ± 15.2 (15.6–91.5)
FEV <sub>1</sub> (L)	0.85 ± 0.45 (0.22–2.14)
FEV <sub>1</sub> (%predicted)	32.8 ± 17.0 (9.1–77.5)
DLco (%predicted)	24.7 ± 11.2 (0.7–58.3)
DLco/VA (%predicted)	24.1 ± 13.6 (0.3–73.6)
Six minutes walking test (range)	
Distance (m)	252.0 ± 89.4 (58–440)
Lowest SpO <sub>2</sub> (%)	84.7 ± 5.9 (67–96)
Arterial blood gas (room air, N = 71) *	
pH (range)	7.42 ± 0.02 (7.37–7.47)
PaO <sub>2</sub> (Torr) (range)	55.7 ± 8.7 (31.6–73.4)
PaCO <sub>2</sub> (Torr) (range)	39.0 ± 5.3 (29.4–54.3)

Plus-minus data are presented as means ± SD.

\* Of 98 LAM patients, 71 had the data of arterial blood gas analysis at room air.

Abbreviations used are: DL<sub>CO</sub>, carbon monoxide diffusing capacity; DL<sub>CO</sub>/VA, DL<sub>CO</sub> per alveolar gas volume; FEV<sub>1</sub>, forced expiratory volume in one second; FVC, forced vital capacity; and %predicted, a percentage of the predicted values.

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## Outcomes of lung transplantation

The survival rate of 57 transplanted patients was 86.7% at 1 year, 82.5% at 3 years, 73.7% at 5 years, and 73.7% at 10 years (Fig 1). Median follow-up duration from transplantation was 1,085 days (S3 Table). Two patients were re-transplanted for graft failure at 38 months or 73 months after the first transplantation. Eleven (20%) of 57 recipients had died at a median of 237 days after transplantation; six patients died of lung infection, four died of graft failure, and two died of malignant neoplasm.

Survival of all 98 LAM patients after registration was 93.5% at 1 year, 85.9% at 3 years, 81.4% at 5 years, and 65.7% at 10 years (S2 Fig); the patients who had lung transplants were included without censoring. When we compared the LAM patients bearing lung transplants ( $n = 57$ ) with those awaiting the transplant opportunity ( $n = 41$ ), the statistical analysis showed no statistically significant difference in the post-registration survival ( $p = 0.053$ ); 94.7% survived at 1 year, 91.1% at 3 years, 84.9% at 5 years, and 73.0% at 10 years vs. the waiting group's 91.8%, 75.6%, 75.6%, and 33.6%, respectively, at each corresponding year (S2 Fig). However, we hypothesize that with larger numbers of patients, a statistically significant difference in survival may be detected since  $p$ -value is so close to the significant level.

The following LAM-related complications were noted within one year after transplantation: pneumothorax of native lung in seven patients (12%) and newly developed chylothorax or chylous ascites in 12 (21%) and four patients (7%), respectively. As compared with pneumothorax, chylous effusion tended to appear earlier after transplantation, presenting in some patients within one week. The recurrence of LAM in the transplanted lung, which was histologically diagnosed by transbronchial lung biopsy (TBLB), was found in four patients (Table 4). In this study, no procedural data were collected such as intra-operative bleeding, operation time, operation-related complications, etc. during transplantation.

## Clinical features of LAM patients with a history of inactive status

We then analyzed the clinical features of LAM patients ( $n = 21$ ) who had a history of inactive status while on the waiting list or were inactive as of March 2014 and compared them with

**Table 2. Haemodynamic data at registration for lung transplantation**

Variable	Value
Echocardiography-n (%)	
Estimated SPAP $\geq 40$ mmHg	16 (16)
Estimated SPAP $\geq 50$ mmHg	3 (3)
Right heart catheterization ( $n = 25$ )	
Heart rate (beats per min)	78.9 $\pm$ 13.9 (61–109)
Mean right atrial pressure (mmHg)	4.9 $\pm$ 2.8 (1–12)
SPAP (mmHg)	31.5 $\pm$ 9.4 (20–50)
MPAP (mmHg)	22.8 $\pm$ 6.8 (13–35)
Patients with $\geq 20$ mmHg-n (%)	12 (48)
Patients with $\geq 25$ mmHg-n (%)*	9 (36)
Mean PCWP (mmHg)	8.1 $\pm$ 4.0 (2–20)
Pulmonary vascular resistance (dyne sec/cm <sup>5</sup> )	256.0 $\pm$ 89.0 (119.0–442.9)
Cardiac index (L/min/m <sup>2</sup> )	3.2 $\pm$ 0.7 (2.0–5.1)

Plus-minus data are presented as means  $\pm$  SD.

\* Of 9 LAM patients, eight patients had mean PCWP  $< 15$  mmHg.

Abbreviations used are: MPAP, mean pulmonary artery pressure; SPAP, systolic pulmonary artery pressure; and PCWP, pulmonary capillary wedge pressure.

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**Table 3. The status of 98 LAM patients who were registered for lung transplantation (as of March 2014).**

Status	
Patients who had been transplanted -n (%)	57 (58)
Age at registration -yr (range)	39.0 ± 7.8 (24–59)
Age at lung transplantation -yr (range)	41.8 ± 8.2 (24–61)
Days from registration to transplantation -median (range)	969 (29–3,381)
History of inactive status on waiting list -n (%)	8 (14)
days of inactive on waiting list -median (range)	170 (48–1,624)
Transplantation	
Deceased-donor lung transplantation—n (%)	53 (93)
Single / Bilateral	48 (91) / 5 (9)
Living-donor lung transplantation—n (%)	4 (7)
Patients on waiting list -n	32 (33)
Active on waiting list—n (%)	20 (20)
History of inactive on waiting list—n (%)	0
Cumulative days from registration -median (range) *	414 (96–1,179)
Inactive on waiting list—n (%)	12 (12)
Cumulative days from registration -median (range)*	2,153 (942–3,461)
Patients who died during waiting term	9 (9)
Days from registration -median (range)	313 (132–3,121)
History of inactive status on waiting list—n (%)	1 (1) **
Cause of death	
Respiratory failure	8 (8)
Hemoptysis	1 (1)

\* Median days from registration to March 2014

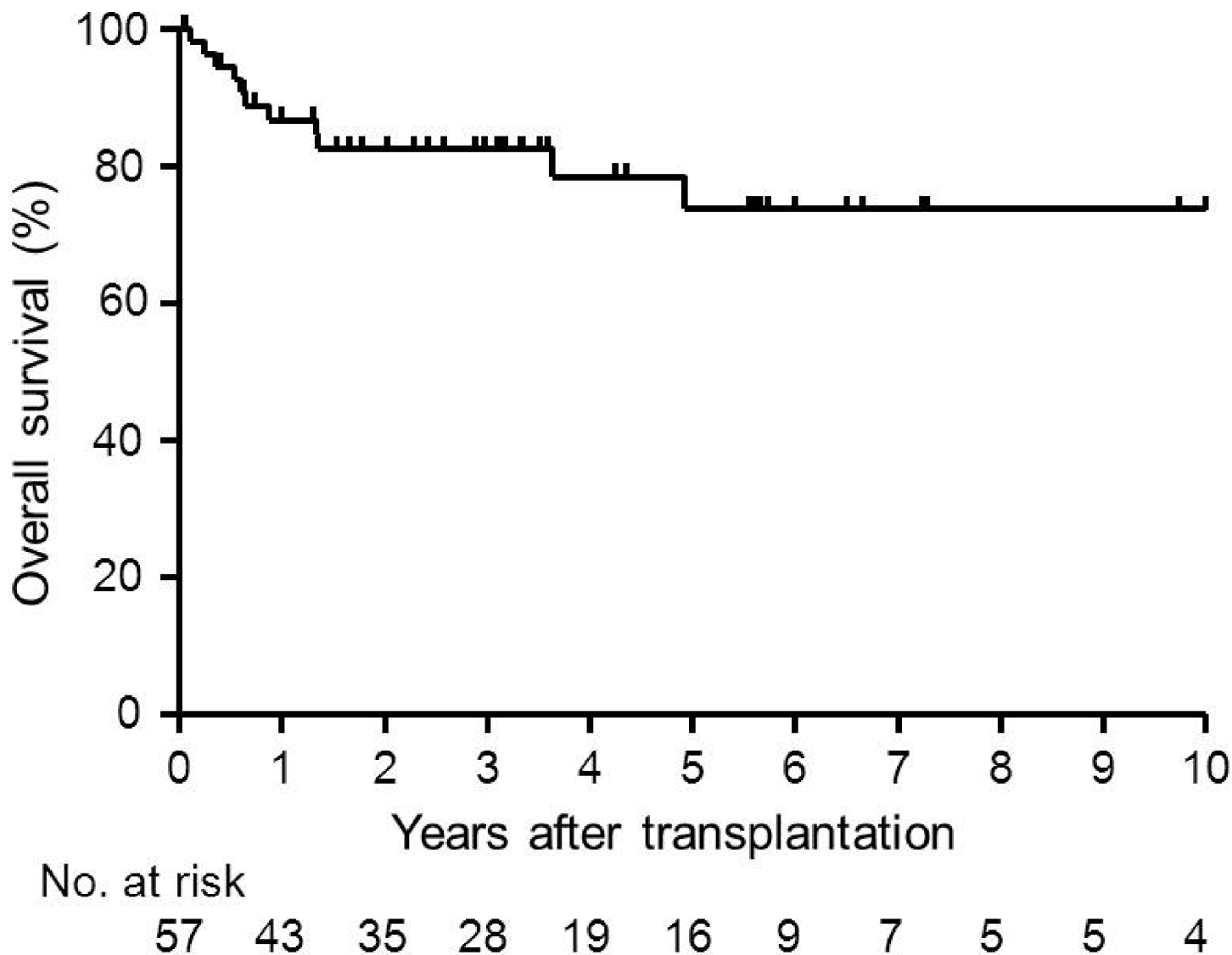
\*\* The patient who died 3,121 days after registration had a history of 1,598 days of inactive status during on the waiting list. If this patient was excluded from this analysis, the median days from registration was 308 and the range was 132–1,816 days.

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those of LAM patients never self-determined in an inactive status ( $n = 77$ ). There was no statistically significant difference in age, results of pulmonary function tests, 6MWD, or results of arterial blood gas analysis. However, patients with an inactive history were more frequently treated with sirolimus than those without an inactive history (67% vs. 5%,  $p < 0.001$ ) (S4 Table).

Of twenty-one patients with an inactive history, eight had already received the transplantation, but the remainders were still inactive as of March 2014. One patient died of respiratory failure during waiting term (Table 3). She had been inactive because of anxiety and fear about operation, but returned to be active after 1,598 days of inactive status because of deterioration of her respiratory condition. However, she had no opportunity of receiving transplantation. Of the eight patients given lung transplants, five had a history of sirolimus treatment while on the waiting list (Fig 2A); three (cases 1 and 7, placebo group; case 2, sirolimus group) participated in the MILES trial [10] and five (cases 2, 3, 4, 5, and 7) were on the off-label use of sirolimus; we could not confirm the period of time in case 3. However, their respiratory conditions had not been stabilized, and then they returned to be active. Three patients (cases 3, 6, and 8) became inactive because of their comorbidities (psychiatric disorders [ $n = 2$ ], thyroid tumor [ $n = 1$ ]). Meanwhile, nine of the twelve patients who retained the inactive status as of March 2014 began the treatment with sirolimus before or during the wait-list period, and all these patients had continued the treatment thereafter. Their respiratory conditions have improved





**Fig 1. Kaplan-Meier calculated survival rate after lung transplantation for 57 patients with LAM.** The median follow-up period was 1,085 days. The probability of survival at 1, 3, 5 and 10 years after lung transplantation was 86.7%, 82.5%, 73.7% and 73.7%, respectively.

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or been stabilized while on sirolimus and then they have still stayed inactive. The remaining three patients without a history of sirolimus have been inactive because of anxiety and fear about operation; one patient participated in the MILES trial, but she was in the placebo group.

## Discussion

Our study, the first national survey of lung transplantation for LAM patients in Japan, revealed that this group's survival rate after transplantation was more favorable than that of patients with other indications [7, 8] and also better than recorded in the registry of The International Society for Heart and Lung Transplantation [7]. The better survival statistics of LAM patients after transplantation was previously proven in four multi-center studies and two single-center studies [6, 14–19] (Table 4). Estenne et al. first reported successful lung transplantation for a patient with LAM in 1984 [14]. Thereafter, Boehler et al. described a retrospective multicenter

series in 1996 but indicated that disease-related complications were frequent [6]. We found that the age at onset of LAM, diagnosis of LAM, or lung transplantation,  $DL_{CO}$  %predicted,  $PaO_2$ , and 6MWD at registration were quite similar among these studies. However,  $FEV_1$  % predicted at registration appears to be better and the waiting period was longer in our population than in those other multi-center studies. The latter issue, the longer waiting time until transplantation, is worthy of special note. This delay was caused primarily by a shortage of donor lungs in Japan. However, an important indicator from this survey is that LAM patients, even those at an advanced stage of disease, can endure until the opportunity for transplantation arrives. Moreover, a favorable survival time is likely once they receive the transplanted lung. This notion is further supported by the fact that the number of LAM patients who died while on the waiting list was only nine (9%).

The occurrences of LAM-related complications in our survey were also similar to those of other studies. In three multi-center studies, the incidences of pneumothorax in native lungs, chylothorax and ascites were 7–14%, 7–14%, and 2%, respectively [6, 16, 17]. We found these complications in 12%, 21% and 7% of patients. The recurrence of LAM in donor lungs is a peculiar post-transplant complication of LAM and also resembled that in other studies (Table 4). The cumulative results of our study confirm that lung transplantation is a satisfactory therapeutic approach for end-stage LAM in Japan, as also found in multiple countries, and this strategy has prolonged life even though LAM is a neoplastic disease.

**Table 4. Summaries of studies on lung transplantation for LAM**

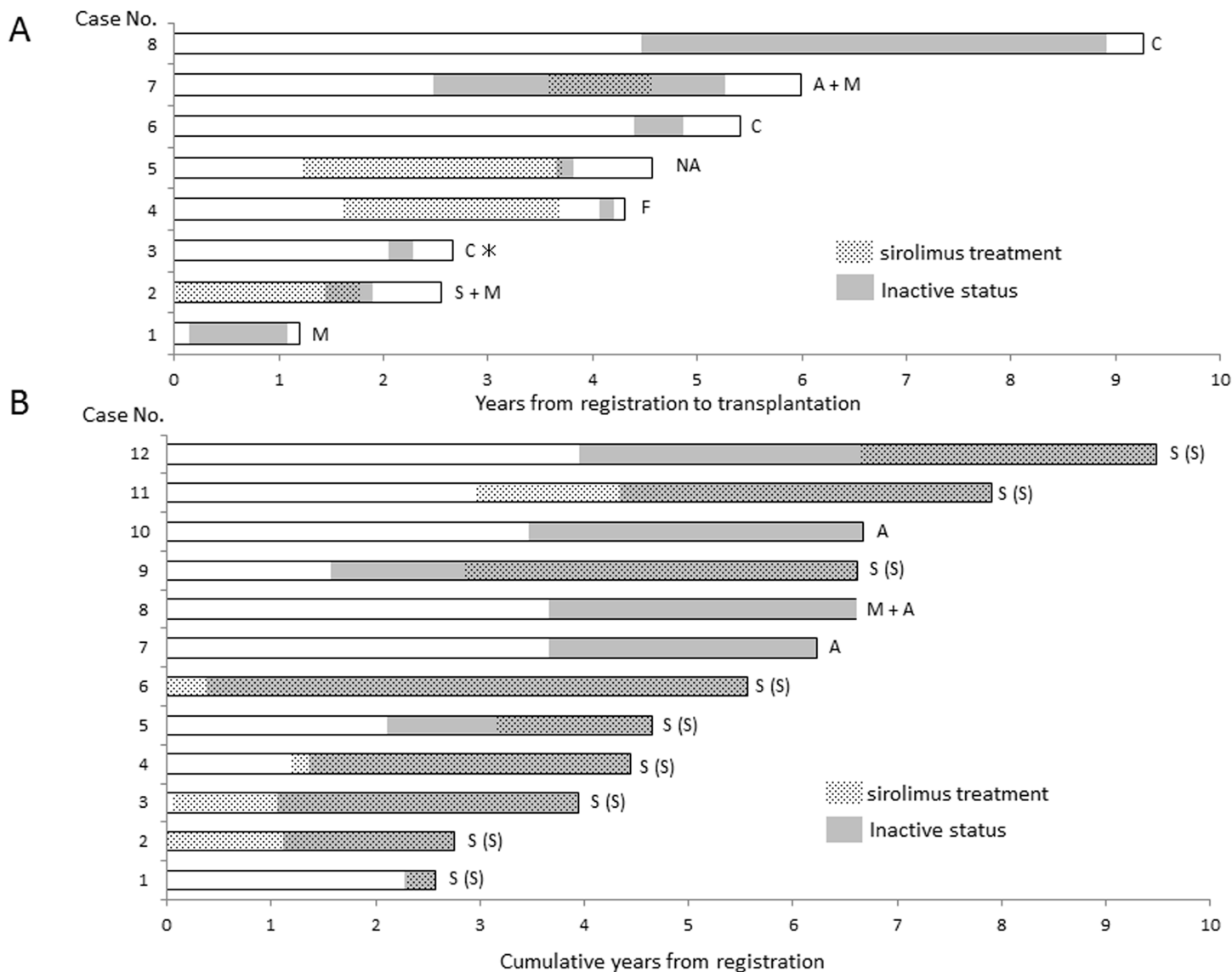
Study	Multi-center studies					Single-center studies	
	Boehler A, et al. <sup>6)</sup>	Kpodonu J, et al. <sup>15)</sup>	Reynaud-Gaubert M, et al. <sup>16)</sup>	Benden C, et al. <sup>17)</sup>	Ando K, et al. (This study)	Pechet TT, et al. <sup>18)</sup>	Machuca TN, et al. <sup>19)</sup>
Country	Switzerland	U.S.	France	Europe	Japan	U.S.	Brazil
Period	1992 to 1995	1987 to 2002	1988 to 2006	1997 to 2007	2000 to 2014	1989 to 2001	1989 to 2009
No. of patients	34	79	44	61	57	14	10
No. of centers	16	31	9	21	6	1	1
Age at onset (mean)	29	-	33	-	31.9	-	-
Age at diagnosis (mean)	34	-	36	34.4	34.0	35.7	-
Age at transplan-tation (mean)	40	41.1	41	41.3	41.8	41.8	43.8
$FEV_1$ %pred (mean)	24	-	22.8	27	32.7	20	32.9
$DL_{CO}$ %pred (mean)	26	-	27.2 <sup>a</sup>	26	24.7	23	38
$PaO_2$ (mean), mmHg	56	-	52.8	(59.3) <sup>b</sup>	54.2	53.7	-
6MWD (mean), m	-	-	214	220	248	(251) <sup>c</sup>	-
Mean waiting time (SD)	-	448 days (322)	-	-	1,060 days (649)	1.9 year (1.0)	-
Survival rate	69% (1y) 58% (2y)	85.8% (1y) 76.4% (3y) 64.9% (5y)	79.6% (1y) 74.4% (2 y) 64.7% (5y) 52.4% (10y)	79% (1y) 73% (3y)	86.7% (1y) 82.5% (3y) 73.7% (5y) 73.7% (10y)	100% (1y) 90% (2y) 69% (5y)	90% (1y) 80% (3y)
Recurrence of LAM (No. of patients)	1	-	2	4	4	1	-

<sup>a</sup> the value is  $DL_{CO}$  /VA %pred.

<sup>b</sup>7.9 kPa or

<sup>c</sup>826 ft was indicated in each report, respectively.

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**Fig 2. Illustration of the periods of inactive status and sirolimus treatment while on the transplantation waiting list.** A. Eight of 21 LAM patients with a history of inactive status had received lung transplants. Five of eight patients had a period during which they had been on the sirolimus treatment (we could not confirm the period of time in case 3\*). The reason why a patient had been inactive is indicated at the right end of each illustration by: A, anxiety and fear about transplantation; C, comorbidity (cases 6 and 8, psychiatric disorder and case 3, thyroid tumor); F, family matter; M, participated in the MILES trial; NA, not available; and S, on sirolimus treatment. Although the cases 1 and 7 participated in the MILES trial, they were in the placebo group; the case 7 took sirolimus after the MILES trial. The clinical courses of cases 2, 4, 5, and 7 who took sirolimus, but their clinical courses were not stabilized sufficiently. B. Of twelve patients who were in an inactive status as of March 2014, nine initiated sirolimus treatment before registration (cases 2 and 6) or while on a waiting list (cases 3, 4, 5, 8, 9, 11, and 12). The reason for being inactive is presented with the same manner as in Fig 2A (A and M); S(S) indicates that a patient has been on sirolimus treatment and respiratory condition has improved or been stabilized.

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We estimated that the prevalence of PH at registration for transplantation would be 16 (16%) of 98 LAM patients in our survey judging by echocardiography (SPAP  $\geq 40$  mmHg) based on the correlation between the results of RHC (MPAP  $\geq 25$  mmHg) and of echocardiography (SPAP  $\geq 40$  mmHg). Taveira-DaSilva et al. reported that the estimated SPAP on echocardiography  $> 35$  mmHg was observed in less than 10% of 95 LAM patients [20], but FEV<sub>1</sub>%predicted and PaO<sub>2</sub> in their subjects were 71.4% and 78.4 Torr, respectively, values that were much milder than those in our population (32.8% and 55.7 Torr, respectively). Our

previous quantitative CT analysis demonstrated that cystic lung destruction did not necessarily accompany a concomitant loss of pulmonary vasculature in LAM [21]. Overall, it would be fair to say that PH is prevalent in less than 20% of LAM patients at their registration for lung transplantation.

The MILES trial proved that sirolimus can inhibit the decline of pulmonary function and stabilize the disease in LAM patients whose FEV<sub>1</sub> is less than 70% of the predicted (mean,  $45 \pm 10\%$  of the predicted) [10]. This is likely to be true in patients with advanced LAM who are prospective candidates for lung transplantation. Our study revealed that sirolimus treatment significantly alleviated the disease course in LAM patients with a history of inactive status ( $p < 0.001$ ) as compared to those without such a history. Additionally, patients who had received sirolimus therapy ( $n = 18$ ) might have prolonged survival after registration compared to those who had never received sirolimus ( $p = 0.073$ , S2 Fig). These data suggest that sirolimus would have a role in stabilizing disease even in end-stage LAM patients. Furthermore, several case reports in the literature document the beneficial role of sirolimus in post-transplant management of LAM patients; sirolimus ameliorates the post-operative chylothorax [22, 23], extra-pulmonary manifestations [24], and recurrence of LAM [25, 26]. In this context, the use of sirolimus is likely to prevail for patients with LAM in need of lung transplantation as a contributor to better prognosis. In addition, sirolimus may have a role in the management of LAM-related complications that occur in the post-transplantation period.

The current uncertainty on the use of sirolimus is how to manage LAM patients who are active candidate for transplants and taking sirolimus. Since bronchial anastomosis dehiscence, a potentially fatal complication of lung transplantation, is reported when a sirolimus-based immunosuppressive therapy was initiated immediately after transplantation [27, 28]. In our cohort, six of 57 transplants (4 with inactive history) was on sirolimus treatment (average, 21 months; range 7 to 37 months) while awaiting transplantation, but were free of sirolimus before transplantation (average, 22 months; range 7 to 43 months). No occurrence of bronchial anastomosis dehiscence was documented in our cohort. At present, all lung transplantation centers in Japan consider to be mandatory for LAM patients to quit taking sirolimus prior to lung transplantation, although evelorimus, another mTOR inhibitor, may be safe to be administered up until lung transplantation [29].

In conclusion, our national survey has revealed that the prognosis after registration as well as that after lung transplantation are favorable in LAM. Lung transplantation is a satisfactory therapeutic option for those with advanced LAM, and the greater use of sirolimus is expected to improve the circumstances of pre-transplantation life for patients with LAM.

## Supporting Information

**S1 Fig. The correlation between SPAP estimated by echocardiography and MPAP measured by RHC ( $n = 13$ ).** They were positively correlated ( $r = 0.589$ ,  $p = 0.034$ ). Six patients whose estimated SPAP was  $\geq 40$  mmHg had MPAP  $\geq 25$  mmHg by RHC. (TIF)

**S2 Fig. Kaplan–Meier calculated survival rate after registration.** A. Survival of all 98 LAM patients after registration was 93.5% at 1 year, 85.9% at 3 years, 81.4% at 5 years, and 65.7% at 10 years (solid line); transplantation is not censored. Survival rates of 57 LAM patients who had lung transplantation (coarsely-dotted line) were 94.7%, 91.1%, 84.9%, and 73.0%, whereas those of 41 patients not transplanted (finely-dotted line) were 91.8%, 75.6%, 75.6% and 33.6% at each corresponding year. B. The solid line indicates survival rates after registration of 18 LAM patients: includes those who had sirolimus treatment while waiting for transplantation ( $n = 9$ ) and also those who had received sirolimus as of March 2014 ( $n = 9$ ). No patients died

while on the waiting list. Of 80 LAM patients who had never received sirolimus, survival after registration was 95.7% at 1 year, 87.5% at 3 years and 78.7% at 5 years ( $n = 80$ , dotted). Recipients of sirolimus tended to have better survival rates after registration than those without sirolimus treatment ( $p = 0.073$ ).

(TIF)

**S1 Table. Clinical characteristics of 98 LAM patients at the diagnosis of LAM.**

(DOCX)

**S2 Table. Correlation between MPAP and other clinical parameters.**

(DOCX)

**S3 Table. Outcomes of 57 LAM patients with lung transplantation.**

(DOCX)

**S4 Table. Comparison between patients with and without a history of inactive status.**

(DOCX)

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1. Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan
2. First Department of Medicine, Hokkaido University School of Medicine, Sapporo, Japan
3. Bioscience Medical Research Center, Niigata University Medical and Dental Hospital, Niigata, Japan
4. Department of Respiriology, Graduate School of Medicine, Chiba University, Chiba, Japan
5. Division of Respiratory Medicine, Juntendo University Faculty of Medicine and Graduate School of Medicine, Tokyo, Japan
6. Department of Sleep and Respiratory Medicine, Nihon University School of Medicine, Tokyo, Japan
7. Division of Pulmonary Medicine, Department of Medicine, Keio University School of Medicine, Tokyo, Japan
8. Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
9. First Department of Medicine, Tokyo Women's Medical University School of Medicine, Tokyo, Japan
10. First Department of Internal Medicine, Shinshu University School of Medicine, Nagano, Japan
11. Department of Respiratory Medicine and Allergy, Tosei General Hospital, Aichi, Japan
12. Department of Thoracic Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan
13. Department of Respiratory Care and Sleep Control Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan
14. Department of Molecular Behavioral Biology, Osaka Bioscience Institute, Osaka, Japan

15. Department of Cardiovascular Medicine, National Cardiovascular Center, Osaka, Japan
16. Clinical Research Center, NHO Kinki-Chuo Chest Medical Center, Osaka, Japan
17. Department of Respiratory Medicine, Shiga University of Medical Science, Otsu, Japan.
18. Second Department of Internal Medicine, Nara Medical University, Nara, Japan
19. Department of Hematology and Respiratory Medicine, Kochi University, Kochi, Japan
20. Division of Respiriology, Neurology, and Rheumatology, Department of Internal Medicine, Kurume University School of Medicine, Fukuoka, Japan
21. Department of Respiratory Medicine, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

\*A lead author for this group, contact e-mail address: [kseyama@juntendo.ac.jp](mailto:kseyama@juntendo.ac.jp)

## Author Contributions

Conceived and designed the experiments: YI TH KS MM. Performed the experiments: YO MA T. Kondo T. Kawamura MO FC HD TS AI NY TN MC. Analyzed the data: KA KS. Contributed reagents/materials/analysis tools: YO MA T. Kondo T. Kawamura MO FC HD TS AI NY TN MC. Wrote the paper: KA YO KS.

## References

1. Johnson SR, Cordier JF, Lazor R, Cottin V, Costabel U, Harari S, et al. Review Panel of the ERS LAM Task Force. European Respiratory Society guidelines for the diagnosis and management of lymphangioleiomyomatosis. *Eur Respir J*. 2010; 35: 14–26. doi: [10.1183/09031936.00076209](https://doi.org/10.1183/09031936.00076209) PMID: [20044458](https://pubmed.ncbi.nlm.nih.gov/20044458/)
2. Carsillo T, Astrinidis A, Henske E. Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. *Proc Natl Acad Sci USA*. 2000; 97: 6085–6090. PMID: [10823953](https://pubmed.ncbi.nlm.nih.gov/10823953/)
3. Kwiatkowski DJ, Zhang H, Bandura JL, Heiberger KM, Glogauer M, el-Hashemite N, et al. A mouse model of TSC1 reveals sex-dependent lethality from liver hemangiomas, and up-regulation of p70S6 kinase activity in Tsc1 null cells. *Hum Mol Genet* 2002; 11: 525–534. PMID: [11875047](https://pubmed.ncbi.nlm.nih.gov/11875047/)
4. Goncharova EA, Goncharov DA, Eszterhas A, Hunter DS, Glassberg MK, Yeung RS, et al. Tuberin regulates p70 S6 kinase activation and ribosomal protein S6 phosphorylation. A role for the TSC2 tumor suppressor gene in pulmonary lymphangioleiomyomatosis (LAM). *J Biol Chem* 2002; 277: 30958–30967. PMID: [12045200](https://pubmed.ncbi.nlm.nih.gov/12045200/)
5. Hayashida M, Seyama K, Inoue Y, Fujimoto K, Kubo K. Respiratory Failure Research Group of the Japanese Ministry of Health, Labor, and Welfare. The epidemiology of lymphangioleiomyomatosis in Japan: a nationwide cross-sectional study of presenting features and prognostic factors. *Respirology*. 2007; 12: 523–530. PMID: [17587419](https://pubmed.ncbi.nlm.nih.gov/17587419/)
6. Boehler A, Speich R, Russi EW, Weder W. Lung transplantation for lymphangioleiomyomatosis. *N Engl J Med*. 1996; 335: 1275–1280. PMID: [8857007](https://pubmed.ncbi.nlm.nih.gov/8857007/)
7. Yusen RD, Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, et al. The registry of the international society for heart and lung transplantation: Thirtieth adult lung and heart-lung transplant report-2013; focus theme: Age. *J Heart Lung Transplant*. 2013; 32: 965–978. doi: [10.1016/j.healun.2013.08.007](https://doi.org/10.1016/j.healun.2013.08.007) PMID: [24054805](https://pubmed.ncbi.nlm.nih.gov/24054805/)
8. Sato M, Okada Y, Oto T, Minami M, Shiraishi T, Nagayasu T, et al. Registry of the Japanese Society of Lung and Heart-Lung Transplantation: official Japanese lung transplantation report, 2014. *Gen Thorac Cardiovasc Surg* 2014; 62: 594–601. doi: [10.1007/s11748-014-0418-6](https://doi.org/10.1007/s11748-014-0418-6) PMID: [24842125](https://pubmed.ncbi.nlm.nih.gov/24842125/)
9. Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell*. 2010; 40: 310–322. doi: [10.1016/j.molcel.2010.09.026](https://doi.org/10.1016/j.molcel.2010.09.026) PMID: [20965424](https://pubmed.ncbi.nlm.nih.gov/20965424/)
10. McCormack FX, Inoue Y, Moss J, Singer LG, Strange C, Nakata K, et al. National Institutes of Health Rare Lung Diseases Consortium; MILES Trial Group. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. *N. Engl. J. Med*. 2011; 364: 1595–1606. doi: [10.1056/NEJMoa1100391](https://doi.org/10.1056/NEJMoa1100391) PMID: [21410393](https://pubmed.ncbi.nlm.nih.gov/21410393/)
11. Ando K, Kurihara M, Kataoka H, Ueyama M, Togo S, Sato T, et al. The efficacy and safety of low-dose sirolimus for treatment of lymphangioleiomyomatosis. *Respir Investig* 2013; 51: 175–183. doi: [10.1016/j.resinv.2013.03.002](https://doi.org/10.1016/j.resinv.2013.03.002) PMID: [23978644](https://pubmed.ncbi.nlm.nih.gov/23978644/)



12. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Respir J* 2009; 34: 1219–1263. doi: [10.1183/09031936.00139009](https://doi.org/10.1183/09031936.00139009) PMID: [19749199](https://pubmed.ncbi.nlm.nih.gov/19749199/)
13. Committee of Respiratory Physiology in Japanese Respiratory Society. Guideline of respiratory function tests—spirometry, flow-volume curve, diffusion capacity of the lung. *Nihon Kokyuki Gakkai Zasshi* 2004 suppl.; 1–56 (Japanese).
14. Estenne M, de Francquen P, Wellens F, Leclerc JL, Vanderhoeft P, Yernault JC, et al. Combined heart-and-lung transplantation for lymphangioleiomyomatosis. *Lancet*. 1984; 1(8371): 275.
15. Kpodonu J, Massad MG, Chaer RA, Caines A, Evans A, Snow NJ, et al. The US experience with lung transplantation for pulmonary lymphangioleiomyomatosis. *J Heart Lung Transplant*. 2005; 24: 1247–1253. PMID: [16143241](https://pubmed.ncbi.nlm.nih.gov/16143241/)
16. Reynaud-Gaubert M, Mornex JF, Mal H, Treilhard M, Dromer C, Quéant S, et al. Lung transplantation for lymphangioleiomyomatosis: the French experience. *Transplantation*. 2008; 86: 515–520. doi: [10.1097/TP.0b013e31817c15df](https://doi.org/10.1097/TP.0b013e31817c15df) PMID: [18724219](https://pubmed.ncbi.nlm.nih.gov/18724219/)
17. Benden C, Rea F, Behr J, Corris PA, Reynaud-Gaubert M, Stern M, et al. Lung transplantation for lymphangioleiomyomatosis: the European experience. *J Heart Lung Transplant*. 2009; 28: 1–7. doi: [10.1016/j.healun.2008.09.014](https://doi.org/10.1016/j.healun.2008.09.014) PMID: [19134523](https://pubmed.ncbi.nlm.nih.gov/19134523/)
18. Pechet TT, Meyers BF, Guthrie TJ, Battafarano RJ, Trulock EP, Cooper JD, et al. Lung transplantation for lymphangioleiomyomatosis. *J Heart Lung Transplant*. 2004; 23: 301–308. PMID: [15019639](https://pubmed.ncbi.nlm.nih.gov/15019639/)
19. Machuca TN, Losso MJ, Camargo SM, Schio SM, Melo IA, Hochhegger B, et al. Lung transplantation for lymphangioleiomyomatosis: single-center Brazilian experience with no chylothorax. *Transplant Proc*. 2011; 43: 236–238. doi: [10.1016/j.transproceed.2010.12.051](https://doi.org/10.1016/j.transproceed.2010.12.051) PMID: [21335196](https://pubmed.ncbi.nlm.nih.gov/21335196/)
20. Taveira-DaSilva AM, Hathaway OM, Sachdev V, Shizukuda Y, Birdsall CW, Moss J. Pulmonary artery pressure in lymphangioleiomyomatosis: an echocardiographic study. *Chest*. 2007; 132: 1573–8. PMID: [17890459](https://pubmed.ncbi.nlm.nih.gov/17890459/)
21. Ando K, Tobino K, Kurihara M, Kataoka H, Doi T, Hoshika Y, et al. Quantitative CT analysis of small pulmonary vessels in lymphangioleiomyomatosis. *Eur J Radiol* 2012; 81: 3925–3930. doi: [10.1016/j.ejrad.2012.05.033](https://doi.org/10.1016/j.ejrad.2012.05.033) PMID: [22964373](https://pubmed.ncbi.nlm.nih.gov/22964373/)
22. Ohara T, Oto T, Miyoshi K, Tao H, Yamane M, Toyooka S, et al. Sirolimus ameliorated post lung transplant chylothorax in lymphangioleiomyomatosis. *Ann Thorac Surg*. 2008; 86: e7–8 doi: [10.1016/j.athoracsur.2008.07.062](https://doi.org/10.1016/j.athoracsur.2008.07.062) PMID: [19021963](https://pubmed.ncbi.nlm.nih.gov/19021963/)
23. Hussein M, Aljehani YM, Nizami I, Saleh W. Successful management of bilateral refractory chylothorax after double lung transplantation for lymphangioleiomyomatosis. *Ann Thorac Med*. 2014; 9: 124–126. doi: [10.4103/1817-1737.128862](https://doi.org/10.4103/1817-1737.128862) PMID: [24791177](https://pubmed.ncbi.nlm.nih.gov/24791177/)
24. Morton JM, McLean C, Booth SS, Snell GI, Whitford HM. Regression of pulmonary lymphangioleiomyomatosis (PLAM)-associated retroperitoneal angiomyolipoma post-lung transplantation with rapamycin treatment. *J Heart Lung Transplant*. 2008; 27: 462–465. doi: [10.1016/j.healun.2008.01.005](https://doi.org/10.1016/j.healun.2008.01.005) PMID: [18374885](https://pubmed.ncbi.nlm.nih.gov/18374885/)
25. Sugimoto R, Nakao A, Yamane M, Toyooka S, Okazaki M, Aoe M, et al. Sirolimus amelioration of clinical symptoms of recurrent lymphangioleiomyomatosis after living-donor lobar lung transplantation. *J Heart Lung Transplant*. 2008; 27: 921–924. doi: [10.1016/j.healun.2008.05.012](https://doi.org/10.1016/j.healun.2008.05.012) PMID: [18656809](https://pubmed.ncbi.nlm.nih.gov/18656809/)
26. Chen F, Omasa M, Kondo N, Fujinaga T, Shoji T, Sakai H, et al. Sirolimus treatment for recurrent lymphangioleiomyomatosis after lung transplantation. *Ann Thorac Surg*. 2009; 87: e6–7. doi: [10.1016/j.athoracsur.2008.07.107](https://doi.org/10.1016/j.athoracsur.2008.07.107) PMID: [19101258](https://pubmed.ncbi.nlm.nih.gov/19101258/)
27. Groetzner J, Kur F, Spelsberg F, Behr J, Frey L, Bittmann I, et al. Airway anastomosis complications in de novo lung transplantation with sirolimus-based immunosuppression. *J Heart Lung Transplant*. 2004; 23: 632–638. PMID: [15135383](https://pubmed.ncbi.nlm.nih.gov/15135383/)
28. King-Biggs MB, Dunitz JM, Park SJ, Kay Savik S, Hertz MI. Airway anastomotic dehiscence associated with use of sirolimus immediately after lung transplantation. *Transplantation*. 2003; 75: 1437–1443. PMID: [12792493](https://pubmed.ncbi.nlm.nih.gov/12792493/)
29. Groetzner J, Kur F, Spelsberg F, Behr J, Frey L, Bittmann I, et al. Airway anastomosis complications in de novo lung transplantation with sirolimus-based immunosuppression. *J Heart Lung Transplant*. 2004; 23: 632–638. PMID: [15135383](https://pubmed.ncbi.nlm.nih.gov/15135383/)